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REVIEW

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Rapid diagnostic tests in the management of pneumonia

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ABSTRACT

Introduction: Pneumonia is one of the main causes of mortality associated with infectious diseases worldwide. Several challenges have been identified in the management of patients with pneumonia, ranging from accurate and cost-effective microbiological investigations, prompt and adequate therapeutic management, and optimal treatment duration.

Areas covered: In this review, an updated summary on the current management of pneumonia patients is provided and the epidemiological issues of infectious respiratory diseases, which in the current pandemic situation are of particular concern, are addressed. The clinical and microbiological approaches to pneumonia diagnosis are reviewed, including discussion about the new molecular assays pointing out both their strengths and limitations. Finally, the current recommendations about antibiotic treatment are examined and discussed depending on the epidemiological contexts, including those with high prevalence of multidrug-resistant bacteria.

Expert opinion: We claim that rapid diagnostic tests, if well-positioned in the diagnostic workflow and reserved for the subset of patients who could most benefit from these technologies, may represent an interesting and feasible tool to optimize timing of targeted treatments especially in terms of early de-escalation or discontinuation of antibiotic therapy.

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1. INTRODUCTION

Pneumonia is among the leading causes of morbidity and mortality worldwide [1]. Several challenges have been identified in the management of patients with pneumonia ranging from accurate and cost-effective microbiological investigations, adequate therapeutic management, and optimal treatment duration.

1.1. Epidemiology and etiology of pneumonia

Defined as acute infection of the lung parenchyma, pneumonia may be caused by a wide variety of microorganisms, including bacteria, viruses, and fungi [2].

Common categories of pneumonia include (i) community-acquired pneumonia (CAP): i.e. an infection acquired outside of the hospital setting, where transmission of pathogens is favored by crowding and close interpersonal contacts (e.g. schools, public recreation facilities/gatherings, public transportation); (ii) hospital-acquired pneumonia (HAP): i.e. an infection acquired in the hospital setting, which emerges at least 48 h after hospital admission; (iii) ventilator-associated pneumonia (VAP): i.e. a subcategory of HAP that occurs in patients receiving mechanical ventilation.

The incidence of CAP varies among studies from 3 to 40 cases/1,000 population per year [3,4], being higher among extreme age categories [4–6] and in the winter period [6,7]. The local behavior regarding vaccination against respiratory pathogens can influence the incidence and severity of the disease in a geographic area [8–10]. Up to 80% of CAP are of mild severity and patients can be managed without hospital admission. The remaining 20% of patients with moderate-severe CAP require hospitalization, with 2–5% of patients needing admission to intensive care unit for severe respiratory failure and/or septic shock [3,4,11]. Mortality rates range from lower than 5% in mild-moderate cases to over 20% in severe cases [3,4,11,12]. Several factors have been associated with mortality among patients with CAP, including older age and comorbidities [13,14], high pneumonia severity index (PSI), concomitant bacteremia, intensive care unit (ICU) admission, and multi-lobar X-ray involvement [15]. CAP morbidity is also relevant: a steady increase in hospitalization rates of patients with CAP has been reported in last years, mainly contributed by elderly patients and those with multiple comorbidities [4,16,17]. Special considerations are needed for immunocompromised patients, as they have a higher risk for CAP [18]. In this group, mixed infections involving viruses, bacteria, and/or fungi have been described [19,20], which represent infrequent events in other populations [21,22]. In addition, the prevalence of antimicrobial resistance in this setting is usually higher than in non-immunocompromised patients [23].

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Article highlights

- Diagnosis and management of patients affected by pneumonia
- Multiple severity scores are recommended for clinical assessment.
- The empirical therapy should be selected based on patient clinical severity, local epidemiology, immune status, and individual risk factors for MDR pathogens.
- Empirical treatments should be revised and possibly narrowed as soon as microbiological results are available.
- Unmet needs still occurring in diagnosis of pneumonia
- The culture of specimens from the lower respiratory tract is currently considered the gold standard in the diagnosis of pneumonia, but it suffers from a significant diagnostic delay and low sensitivity.
- Molecular tests based on NAATs are highly sensitive but can detect a limited number of pathogens.
- Molecular tests based on antigen detection are specific for single pathogens and may suffer from reduced sensitivity.

The main etiological causes of CAP include *Streptococcus pneumoniae, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Haemophilus influenzae, Legionella pneumophila*, anaerobes (in case of aspiration pneumonia), and several respiratory viruses including *Influenza A-B, Parainfluenza, Respiratory Syncytial Virus, Human Metapneumovirus, Coronaviruses, and Rhinoviruses* [11,24,25]. Recently, global health is facing with the new Coronavirus disease 2019 (COVID-19) pandemic caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infection, which has completely transformed the epidemiology of CAP. Indeed, about 11% of COVID-19 patients needed hospitalization and 18% of these needed intensive care support for critical pneumonia [26]. Diagnosis of SARS-CoV-2 pneumonia could be challenging as it may have unspecific clinical and radiological features, especially in the most serious cases. Moreover, coinfection between SARS-CoV-2 and other community pathogens may be possible, although it has been reported in a few percentage of patients (3–8%) compared with those admitted for influenza (up to 25%) [27].

According to recent studies on lung microbiome, lower airways and alveoli are no longer considered a sterile environment, while they are settled by a commensal microbiota mainly composed by anaerobic bacteria like *Prevotella, Veillonella, Fusobacterium*, and by *Haemophilus* and *Streptococci* [28]. An antecedent or simultaneous viral infection may disrupt this microbial homeostasis favoring the overgrowth of bacteria causing CAP [9]. Moreover, some studies suggested that clinical severity of CAP may be determined by interactions between microbiota dysbiosis and the immune system [14]. This revised pathogenetic model may affect current statements on CAP therapeutic management concerning, for example, the role of antibiotics versus reconstitution of normal microbiota or immunomodulation as potential therapeutic target.

The incidence of HAP varies from 5 to 10 cases/1,000 admitted patients, increasing 6–20 folds in mechanically ventilated patients [29]. The rate of VAP is on average 20% among patients requiring mechanical ventilation (MV), with an incidence of 10–15 cases/1,000 MV [30]. Both HAP and VAP are associated with high morbidity and mortality, prolonged length of stay, increased healthcare costs, and a 15–45% attributable mortality [31]. Main factors influencing death for HAP are need of ICU admission and mechanical ventilation, higher Simplified Acute Physiologic Score II (SAPS II), multi-drug resistant (MDR) *Acinetobacter baumannii* or *Pseudomonas aeruginosa* infection, duration of antibiotic treatment, occurrence of acute kidney injury or having underlying hematologic diseases [32]. Similarly, major mortality predictors for VAP were found to be MDR infections, severe sepsis/septic shock, acute respiratory distress syndrome (ARDS), malnutrition, pulmonary complications (i.e. pleural effusions, multi-lobar lung involvement) [33], elevated neutrophil-to-lymphocyte count ratio levels, and high blood urea nitrogen/blood albumin ratio [34]. Studies where the etiology of HAP and VAP was systematically assessed, reported a similar pathogen distribution between the two categories [35,36]. *Staphylococcus aureus*, mainly methicillin-resistant strains, non-fermenting Gram-negative bacteria (mainly *Pseudomonas aeruginosa*) and *Enterobacterales* account for the majority of episodes [31,35,36]. In the last years, an increase in the prevalence of Gram-negative over Gram-positive pathogens has been reported [35]. Concomitantly, the emergence and spread of MDR and extensively drug resistant (XDR) phenotypes among Gram-negative bacteria [37] has made the choice of empirical antibiotic therapy more difficult, bringing to an extensive use of broad-spectrum antibiotics [38,39].

1.2. Management of pneumonia: clinical and laboratory work up

Once the diagnosis of pneumonia is established, based on clinical symptoms and radiological findings, antibiotic treatment should be initiated without delay [40]. Early administration of antibiotic therapy has been considered a standard of care in the management of pneumonia since two retrospective studies showed that receiving the first antibiotic dose within 4–8 h of admission improved survival [41,42]. However, these findings were not confirmed by further prospective studies or meta-analyses [43]. Furthermore, early administration of antibiotics has been criticized by some authors because of the potential for misdiagnosis of pneumonia, the risk of antibiotic overuse, and the possibility of a lower yield in the microbiological workup [44,45]. Thus, more recent guidelines do not specify a time window for delivery of the first antibiotic dose [2,46]. It is worth mentioning that in presence of sepsis with a sequential organ failure assessment (SOFA) score higher than 2 and/or septic shock, guidelines recommend initiating antibiotics within 1 h of diagnosis [47].

Some biomarkers have been assessed for guiding management of antibiotic therapy along with clinical evaluation. Due to its rapid increase in bacterial infections and reduced release in viral infections, serum procalcitonin (PCT) has been considered a promising tool for discriminating between viral or bacterial origin of CAP and guiding the initiation of antibiotic therapy. However, due to inconclusive results from several randomized controlled trials (RCTs), current guidelines recommend against the use PCT to initiate antibiotic therapy in

patients with CAP [46]. In HAP/VAP patients, guidelines recommend the use of PCT just to support withholding antibiotics in patients with a favorable clinical course [38].

The limited yield of PCT in VAP has prompted to search for other markers for pneumonia management. Interleukin (IL)-6 has been recognized an earlier marker in localized infections rather than PCT [48]. It has also been assessed as useful proxy of treatment failure and mortality [49]. In addition, from the beginning of COVID-19 pandemic, IL-6 has received major attention as a possible predictor of severe disease along with a novel target for therapies aimed to decrease inflammatory response [50]. One of the main limits is lack of specificity, since IL-6 rises in a wide range of conditions among immune, hematopoietic and inflammatory diseases [51]. Similarly, kinetic of serum levels of pro-adrenomedullin (a stable pro-hormone produced through degradation of adrenomedullin) was found to predict mortality in CAP after the first 48 hours from antibiotic administration [52].

Table 1 summarizes the recommendations about initial treatment strategies in patients with CAP, HAP and VAP according to the most recent guidelines.

2. MICROBIOLOGICAL DIAGNOSIS OF PNEUMONIA

2.1. Microbiological diagnosis of pneumonia: the state of the art

Most clinicians argue that, in the outpatient setting, microbiological testing is likely to have a low yield and to add little to the choice and duration of antibiotic therapy. Indeed, the low yield of the microbiological workup and the favorable outcome usually obtained with empirical therapy have resulted in an overall low use of microbiological testing for the management of patients with mild CAP [53]. Nevertheless, microbiological workup has been associated with a better outcome among hospitalized patients with CAP [53]. Current guidelines agree that microbiological testing is not mandatory in patients with low-severity CAP, while in cases of moderate- and high-severity CAP and/or in the presence of risk factors for antibiotic resistant pathogens and/or immunosuppression, microbiological testing based on Gram stain and bacteriological culture is strongly recommended [46]. In patients without purulent sputum or who are unable to provide lower respiratory tract samples, culture results can be inaccurate and commonly represent upper airway or oropharyngeal colonizers rather than true pathogens. In these cases, a bronchoalveolar lavage (BAL) can be performed to provide an adequate sample. BAL testing is also recommended for immunosuppressed patients or those with severe CAP failing initial therapy [53]. In case of HAP, including VAP, some guidelines recommend performing Gram stain on respiratory sample as a first step [39,54], but its usefulness is still debated [39], while there is consensus in saying that blood and respiratory cultures are the best way to make etiological diagnosis of pneumonia. Currently the method of sampling (invasive or noninvasive, blind or ultrasound-guided) and the analytic method (qualitative, quantitative or semi-quantitative) are controversial and largely debated because there is no clear evidence or expert consensus supporting that quantitative cultures are associated with better clinical outcomes than qualitative ones, regardless the method used to collect respiratory secretions (invasive or noninvasive) [55].

The culture of specimens from the lower respiratory tract (i.e. bronchial aspirate, BAL) is currently considered the gold standard in the diagnosis of pneumonia and should be forerun by microscopical evaluation of the respiratory sample for assessing the quality of the specimen (i.e. absence of oral epithelial cells and/or presence of immunity cells). Moreover, quantitative or semiquantitative culture based on the bacterial load, even though of controversial clinical impact, can help distinguishing pathogens from contaminants and making a correct diagnosis [53]. However, the culture-based approach has some limitations in terms of low sensitivity and long turnaround time of medical reports. The sensitivity of culture is affected by previous antibiotic exposure, accuracy of sampling procedure, conditions of storage and transport of sample to the laboratory, and also by the non-homogeneous distribution of microorganisms in respiratory fluids [56]. Besides, the culture method entails a significant diagnostic delay, often incremented by the discontinuous laboratory opening time. In most cases this diagnostic delay inevitably requires the initiation of an empiric broad-spectrum antibiotic therapy that may not always be appropriate, possibly leading to an increment of mortality rate and favoring the emergence of multidrug resistance [57]. All efforts should be made to ensure a suitable and timely diagnostic pathway aimed to enhance the diagnostic yield and obtain an etiologic diagnosis, which is necessary for choosing the appropriate treatment and limiting the development of antibiotic resistance [57].

The diagnostic delay associated with cultural tests can be significantly shortened by molecular tests based on the search for microbial antigens by rapid immunoassays or for microbial nucleic acids using amplification tests (NAATs). However, molecular tests based on NAATs can detect only a limited number of pathogens, depending on the probes aboard the tests, and can be used only on selected patients due to their high costs, while those based on antigen detection are cheaper but may suffer from reduced sensitivity and only target some specific pathogens [56,58].

Overall, due to the low sensitivity of culture, and the limited number of microbial targets detectable by molecular assays, a causative pathogen can be detected in less than 40% of adults with CAP [11]. Such a high percentage of false negative results in the microbiological diagnosis of pneumonia has important implications on clinical choices. Indeed, a false negative test could lead clinician to attribute respiratory symptoms to noninfectious inflammatory status and to empirically start corticosteroids-based therapy, which can worsen an occult infective status. Moreover, the lack of trust in the reliability of a negative test can lead clinician to overuse antibiotics [59].

Table 1. Empirical treatment of CAP, HAP, VAP according to the most recent guidelines.

			IDSA-ATS 2019	NICE 2019
CAP	Mild	Without risk factors for MDR	Outpatients: amoxicillin or doxycycline or macrolide; in patients with comorbidities: combination with amoxicillin/clavulanate or cephalosporin and macrolide or doxycycline or monotherapy with respiratory fluoroquinolone Inpatients: beta-lactam + macrolide or fluoroquinolone	Oral amoxicillin (add macrolide if atypical pneumonia suspected) If penicillin allergy/atypical pneumonia suspected: macrolide or doxycicline
		With risk factors for MDR	Obtain cultures/nasal PCR and add MRSA and *P. aeruginosa* coverage only if positive Prior respiratory isolation of MRSA/*P.aeruginosa*: add antibiotic coverage and obtain cultures/nasal PCR to allow descalation or confirmation of therapy	
	Moderate-severe	Without risk factors for MDR	Beta-lactams + macrolide or beta-lactam + fluoroquinolone	Amoxicillin or amoxicillin/clavulanate + macrolide If penicillin allergy: macrolide or levofloxacin
		With risk factors for MDR	Add MRSA/ *P. aeruginosa* coverage and obtain cultures/nasal PCR to allow descalation or confirmation of therapy	
			IDSA 2016	**ERS/ESICM/ESCMID/ALAT 2017**
HAP	Low mortality risk	Without risk factors for MDR	Provide MSSA coverage + 1 antipseudomonal antibiotic	Use narrow-spectrum monotherapy: ertapenem/ceftriaxone/ cefotaxime/ moxifloxacin or levofloxacin
	High mortality risk	With risk factors for MDR	Provide anti-MRSA agent + 2 antipseudomonal antibiotics of different classes	Use combination therapy: single Gram-negative agent (if active >90% GN in the ICU) +- MRSA therapy If septic shock: dual Gram-pseudomonal coverage +- MRSA therapy
VAP	Low mortality risk	Without risk factors for MDR	Provide MSSA coverage + 1 antipseudomonal antibiotic	Use narrow-spectrum monotherapy: ertapenem/ceftriaxone/ cefotaxime/ moxifloxacin or levofloxacin

Due to the aforementioned limitations of conventional microbiologic tests in terms of sensitivity, speed, and spectrum of targets, it is commonly accepted that the implementation of microbiological diagnostics allowing for a rapid and reliable detection of a wide panel of respiratory pathogens is of great interest to antimicrobial stewardship [60].

2.2. Emerging technologies in rapid microbiological diagnosis for pneumonia

In the last decade many efforts have been made to improve the diagnosis of CAP and HAP (including VAP). In particular, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is an easy to use, accurate, and cost-effective technology that has caused a revolutionary shift in clinical microbiology allowing a rapid and accurate identification of cultured microorganisms, and consequently reducing time to identification of bacterial and fungal pathogens [61]. Despite MALDI-TOF MS has considerably streamlined the diagnostic workflow, the conventional culture-based microbiology still remains too slow for optimal antimicrobial stewardship. NAATs and fluorescence microscopy-based technologies represent novel approaches that could significantly improve the care of patients affected by respiratory infections, reducing dramatically the diagnostics delays and allowing clinicians to timely set up a more targeted therapy, improving antimicrobial stewardship and minimizing the use of broad-spectrum antibiotics [58,62,63].

Furthermore, in the last years, the need of simple tools, potentially usable as point of care at patient's bedside, for the rapid, cost-effective, and noninvasive diagnosis of pneumonia has led researchers to carry out interdisciplinary studies assessing the use of chemical and electrochemical analytic techniques as alternative methods for the diagnosis of respiratory diseases [64]. In the field of infectious disease, several studies have been performed so far, especially with regards to lungs infection in cystic fibrosis patients and more recently to SARS-CoV-2 infection, which confirm that certain diseases can result in characteristic changes in volatile organic compounds (VOCs) profiles of the exhaled breath, detectable using conventional gas chromatography-MS and novel nano-composite sensor

arrays (E-nose) [64–68]. Therefore, the analysis of breath fingerprints can be used as a novel noninvasive method to early diagnose and monitor some bacterial and viral infections [64,69]. Even if methods based on exhaled breath analysis are promising in pneumonia diagnosis, there are still several challenges (i.e. variability induced by environmental factors) and limitations (i.e. need of larger scale validation studies to further establish the accuracy of breath analysis) that currently preclude their wide clinical applicability [70].

Nowadays, diagnostic tests based on NAAT technologies are the most commonly used in clinical microbiology because they allow for the rapid detection of bacterial/viral/fungal targets and resistance mechanisms (molecular antibiogram) directly from clinical specimens with high specificity and sensitivity levels. Some molecular systems offer syndromic panels targeting the most common pathogens for specific clinical conditions (e.g. bloodstream infections, meningitis, respiratory tract infections, etc) and are very appealing for clinicians and microbiologists to achieve a microbiological diagnosis in the fastest and simplest way [56]. NAATs have played also a key role in the diagnosis of SARS-CoV-2 infections. Indeed, following the pandemic emergence, a multitude of molecular diagnostic tests (rRT-PCRs, RT-LAMP, TMA, automated and/or multiplex methods based on proprietary platforms) have been developed for the detection and/or quantification of SARS-CoV-2 RNA and are essential for decisions on COVID patient's management, hospital infection prevention practices, and public health responses [71,72]. Due to their sensitivity, in some cases NAATs could also be useful for diagnosis of pneumonia starting from oral washes or other upper respiratory tract specimens. For instance, in clinically unstable and/or immunocompromised individuals where BAL collection may not be safe, NAATs can be carried out on oral washes to make diagnosis of *P. jirovecii* pneumonia. Similarly, NAATs can be used to perform large scale screening of SARS-CoV-2 in oral fluids when nasopharyngeal swabs are difficult to collect, such as in children [73]. Even if the performances of NAATs in detecting pathogens from oral samples when more invasive samples are not easily obtainable are encouraging, attention should be paid in interpretation of these results. Indeed, a positive result may simply represent colonization, as it could happen in case of *P. jirovecii*, while a negative result at the oral level may not exclude a lower respiratory tract infection, as in case of SARS-CoV-2. Therefore, a clinical judgment is always recommended for a correct interpretation of results [73].

Among NAATs, only few CE-IVD labeled and FDA cleared tests are currently available to be used directly on respiratory specimens. The VERIGENE® Respiratory Pathogens Flex Nucleic Acid Test (RP Flex) (Luminex Molecular Diagnostics, Inc., Canada) is an FDA cleared and CE-IVD marked test able to detect 13 viral and 3 bacterial targets (Table 2) in less than 2 h with a declared sensitivity of 79.2–100% and specificity of 97.1–100%, depending on target. The system is highly flexible offering the possibility to use all or selected targets. However, the panel proposed is limited and designed basically for CAP, covering several viral targets and few bacterial pathogens belonging to *Bordetella* genus only, while it does not include some important pathogens involved in CAP such as SARS-CoV-2, *S. pneumoniae, H. influenzae, Mycoplasma pneumoniae*, and *Legionella pneumophila*. Furthermore, the result provided is only qualitative and no information about viral or bacterial abundance are supplied.

During pandemic, the importance of making a rapid differential diagnosis of COVID-19 in patients with acute respiratory infections, has led to the implementation of panels for the diagnosis of CAP including also SARS-CoV-2 targets, such as QIAstat-Dx® Respiratory SARS-CoV-2 (Qiagen GmbH, Germany) and NxTAG®RPP+SARS-CoV-2 (Luminex Molecular Diagnostics, Inc., Canada).

QIAstat-Dx® Respiratory SARS-CoV-2 Panel is a CE-IVD marked, fully automated, multiplex polymerase chain reaction (PCR) assay able to detect 3 bacterial species (*Bordetella pertussis, Mycoplasma pneumoniae*, and *Legionella pneumophila*) and 18 viruses (or their subtypes), including SARS-CoV-2, in nasopharyngeal swabs in UTM™ (Table 2). This system requires a small sample volume and a minimal hands-on time and gives a qualitative result in approximately one hour with a sensitivity declared by the Manufacturer of 85.1–100% and specificity of 97.9–100%, depending on the target.

NxTAG®RPP+SARS-CoV-2 is a CE-IVD multiplex real-time PCR for the qualitative detection of 20 respiratory viruses, including SARS-CoV-2, and 3 atypical bacteria in several clinical samples from upper-airways (i.e. nasopharyngeal, oropharyngeal, nasal, anterior nasal, and mid-turbinate nasal swabs, nasal aspirates, and nasal wash in UTM™, Liquid Amies, or equivalent) (Table 2). This test, developed to be used on a high-throughput system processing up to 96 samples in less than 3 hours (excluded the additional time for the nucleic acids extraction step) with a minimal hands-on time, shows good performances with a positive predictive value declared by the Manufacturer of 88.9–100% and a negative predictive value of 99.5–100%, depending on target.

The BioFire FA Pneumonia Panel Plus (bioMérieux, France) (FA-PPP) is another FDA cleared and CE-IVD marked test able to detect 18 bacteria (3 of which atypical), 8 viruses, and 7 antimicrobial resistance-associated genes (Table 2), directly from any respiratory sample, in about 1 hour with a declared sensitivity of 75–100% for sputum and 85.7–100% for BAL, and specificity of 87.2–99.4% for sputum and 91.2–99.5% for BAL, respectively) [53,74,75]. The wide panel of bacterial and viral targets investigated, make this system very helpful both in CAP and HAP (including VAP) diagnosis and even if it lacks SARS-CoV-2 target, could have also a valuable role in the management of COVID patients, because of the numerous bacterial targets investigated together with its high specificity/sensitivity and its high negative predictive value which allow for an early diagnosis or the exclusion of bacterial coinfections. In comparison to other molecular tests, FA-PPP also provides an estimate of nucleic acid abundance (copies/ml) for 15 different bacterial groups or species. Recent studies demonstrated that nucleic acid abundance reported by FA-PPP is approximately 1 $\log_{10}$ higher than value (CFU/ml) reported from culture [53,74,76]. The quantitative value provided by FA-PPP could potentially give useful information to distinguish infection (when bacterial targets are present at high number of copies/ml) from colonization (when bacterial targets are present at low number of copies/ml) [53].

Table 2. Pathogens and resistance mechanisms targeted by three different CE-IVD marked/FDA-cleared NAATs.

Target	VERIGENE® Respiratory Pathogens Flex Nucleic Acid Test	BioFire® FilmArray®Pneumonia Panel Plus	Curetis Unyvero Hospitalized Pneumonia panel (HPN)	QIAstat-Dx® Respiratory SARS-CoV-2	NxTAG®RPP+SARS-CoV-2
Viral	Adenovirus Human Metapneumovirus Influenza A Influenza A (subtype H1) Influenza A (subtype H3) Influenza B Parainfluenza 1 Parainfluenza 2 Parainfluenza 3 Parainfluenza 4 Rhinovirus RSV A RSV B	Adenovirus Coronavirus Human Rhinovirus/ Enterovirus Human Metapneumovirus Influenza A Influenza B Parainfluenza virus Respiratory Syncytial Virus		Adenovirus Coronavirus 229E Coronavirus HKU1 Coronavirus NL63 Coronavirus OC43 Human Metapneumovirus A/B Influenza A Influenza A subtype H1 Influenza A subtype H3 Influenza A 2009 H1N1 Influenza B Parainfluenza 1 Parainfluenza 2 Parainfluenza 3 Parainfluenza 4 RSV A/B Rhinovirus/Enterovirus SARS-CoV-2	Adenovirus Coronavirus 229E Coronavirus HKU1 Coronavirus NL63 Coronavirus OC43 Human Bocavirus Human Metapneumovirus Influenza A Influenza A subtype H1 Influenza A subtype H3 Influenza A 2009 H1N1 Influenza B Parainfluenza 1 Parainfluenza 2 Parainfluenza 3 Parainfluenza 4 RSV A RSV B Rhinovirus/Enterovirus SARS-CoV-2
Bacterial	*Bordetella pertussis* *Bordetella parapertussis/ Bordetella bronchiseptica* *Bordetella holmesii*	*Acinetobacter calcoaceticus/ baumannii* complex *Enterobacter cloacae* *Escherichia coli* *Haemophilus influenzae* *Klebsiella oxytoca* *Klebsiella pneumoniae group* *Klebsiella aerogenes* *Moraxella catarrhalis* *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens* *Staphylococcus aureus* *Streptococcus agalactiae* *Streptococcus pneumoniae* *Streptococcus pyogenes* *Chlamydophila pneumoniae* (atypical) *Legionella pneumophila* (atypical) *Mycoplasma pneumoniae* (atypical)	*Acinetobacter* spp. *Chlamydophila pneumoniae* *Citrobacter freundii* *Enterobacter cloacae* complex *Klebsiella aerogenes* *Escherichia coli* *Haemophilus influenzae* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Klebsiella variicola* *Legionella pneumophila* *Moraxella catarrhalis* *Morganella morganii* *Mycoplasma pneumoniae* *Proteus spp.* *Pseudomonas aeruginosa* *Serratia marcescens* *Staphylococcus aureus* *Stenotrophomonas maltophilia* *Streptococcus pneumoniae*	*Bordetella pertussis* *Chlamydophila pneumoniae* (atypical) *Mycoplasma pneumoniae* (atypical)	*Chlamydophila pneumoniae* (atypical) *Legionella pneumophila* (atypical) *Mycoplasma pneumoniae* (atypical)
Fungal			*Pneumocystis jirovecii*		
Resistance		*mecA/C* e MREJ KPC NDM OXA-48-like CTXM VIM IMP	*mecA* *mecC* KPC NDM OXA-23 OXA-24/40 OXA-48-like OXA-58 VIM IMP CTXM TEM SHV *ermB* *sul1* GyrA83 GyrA87		

Another NAAT-based system for the rapid diagnosis of pneumonia is the Unyvero system (Curetis GmbH, Germany) (CE-IVD marked/FDA-cleared) that requires a TAT of about 4–5 hours. The Unyvero Hospitalized Pneumonia (HPN) panel offers a wide range of respiratory pathogens including 20 bacterial targets, 1 fungal target, and 17 antibiotic resistance targets, but it lacks viral targets at all (Table 2). The Manufacturer declares that HPN has a good sensitivity (range 80–100%) and high specificity values (range 98–100%) for microbial targets [77]. On the other hand, a poor consistency between resistance marker detection and organisms' antibiograms is reported in performance data sheet, an issue spotted also with the predecessor panel Unyvero P55 that showed a very low sensitivity for antibiotic resistance detection (18%) [76,78]. Despite the good sensitivity and specificity values declared by the Manufacturer for microbial targets [77], recent studies reported lower performances of the Curetis system [79,80]. A study funded by the National Institute for Health Research (NIHR), aimed to compare FA-PPP and HPN, described lower performances of HPN (sensitivity range 50–100%; specificity range 89.4–100%) respect to those declared by Curetis, and pointed out also a higher rate of HPN failed tests (4%) than FA-PPP (1.9%) [79,80]. The authors conclude that, even if both platforms performed overall similarly, FA-PPP revealed a slightly greater sensitivity, fewer major discordances, and fewer machine failures. Besides, a more rapid turnaround time and the smaller size allow for an easier implementation in the diagnostic workflow [74].

Another molecular technology underlies the Accelerate Pheno™ system (Accelerate Diagnostics, Spain), a fully automated system that combines fluorescence in situ hybridization (FISH) for ID and time-lapse microscopy to determine MIC values. This system is FDA approved and CE-IVD marked for the diagnostic of bacteremia and is able to detect 15 bacterial genus or species targets and 2 fungi directly from positive blood culture. Preliminary studies have shown that this system has good performances in terms of sensitivity and specificity (100% and 97%, respectively) also on respiratory samples [62]. However, it is still a prototype and needs to be further developed before being used for the diagnosis of respiratory infections.

2.3. The challenges of implementation of rapid molecular diagnostic microbiology

NAATs have also some drawbacks. As previously mentioned, these technologies are characterized by a limited panel of genetic markers for bacterial/viral/fungal identification and/or resistance mechanisms and may miss novel or rare pathogens not covered by the probes, affecting the overall negative predictive value of the tests [81]. Hence, it is essential to choose the most suitable molecular tests in collaboration with physicians, based on patients clinical status and epidemiological settings and carrying out simultaneously a conventional culture-based investigation [81]. Moreover, the implementation of NAATs in pneumonia diagnosis has brought new challenges in the interpretation of positive results. Indeed, due to the overall higher sensitivity of the molecular tests, a positive result for a certain pathogen by molecular testing might not be confirmed by cultural analysis, while the identification of a resistance gene may not necessarily correlate with the presence of an isolate with the corresponding resistance phenotype (possibly due to a low level or lack of gene expression). Therefore, the implementation of such molecular tests needs an active support to clinicians in the interpretation of the results.

Furthermore, the high costs (about 30–50× the cost of conventional culture) require an appropriate positioning in the laboratory workflow to maximize benefits for patient outcomes by shortening the turnaround time. On the other hand, the economic burden is relative if compared to the healthcare cost of a late diagnosis in terms of patient's outcome and patient's management. A possible strategy to implement a fast microbiology workflow in a feasible way could be reserving these technologies to a subset of patients admitted to critical wards (e.g. ICU and hematology-oncology) or to a subset of patients classified as at high-risk on the basis of a multiparametric approach (i.e. scores, microbiological data, biomarkers) [82]. This diagnostic workflow could produce not only clinical benefits but also substantial economic benefits due to the optimization of resources.

2.4. Data reporting of rapid molecular tests for the diagnosis of pneumonia: a new challenge for the microbiologist

If on one side the new technologies have largely improved microbiological diagnosis in terms of turn-around-time and sensitivity, on the other side they have posed a new challenge to clinical microbiologists regarding the interpretation and the correct communication of results to clinicians, especially when it comes to bacteria detection.

Either when cultural method or molecular diagnostics are adopted, results from lower respiratory tract samples are among the hardest to be interpreted due to the potentially high rate of contamination of these specimens with the microbiota from the upper airways. The contamination issue is closely dependent on the way of sampling (i.e. lower risk of contamination in correctly executed BAL and higher risk of contamination in sputum samples) and requires the microbiologist's skill in distinguishing pathogens from colonizing microorganisms. In this perspective, the dialogue with clinicians could be very useful to microbiologists to know the patient conditions and to correctly interpret the meaning of bacterial target(s) detected. Molecular diagnostics partially overcome this problem because many of the most common upper airway colonizers are not included in the panels (i.e. *viridans streptococci*). However, when bacteria existing both as pathogens and colonizers are detected (e. g., *A. baumannii*), the interpretative issue remains. In these cases, tests providing a semi-quantitative result could have an added value compared to those providing qualitative information only, because they allow to evaluate the possible significance of the detected bacteria as done with the cultural method. In this perspective, FA-PPP provides the abundance of bacterial

target reporting nucleic acid copies/ml from a minimum of 10^4 copies/mL to a maximum of 10^7 copies/mL. However, since these quantitative values provided by FA-PPP are not equivalent to CFU/mL, training clinicians on the differences between culture quantitative values (CFU/ml) and nucleic acid abundance (copies/ml) is fundamental to avoid potential misinterpretations [76].

Another challenge regards the format of data reporting. It is mandatory that all targets investigated by molecular tests are listed in the report with the respective result, so that even a negative result could be informative and helpful in driving clinicians to etiological diagnosis. The more complete and simplest way of reporting should consist in indicating each target as 'detected' or 'not detected.' However, this leads to the production of more complex and detailed reports that require a connection between diagnostic instruments and Laboratory Information Systems to simplify and quicken the step of reporting and to reduce the risk of error.

Another challenge of molecular diagnostics concerns the reporting of resistance mechanisms detection. The simplest and most common condition is when a bacterial species is detected together with a resistance mechanism that is frequently associated with that species. In this case it is recommended to report both targets as '*detected*' adding a note explaining the likely resistance phenotype (e.g. in case of detection of *Klebsiella pneumoniae* and KPC the note could read '*possible resistance to all beta-lactam antibiotics, including carbapenems, except combinations with new KPC inhibitors or cefiderocol*'). The information should be stated as a probability, because the detection of a resistance gene does not necessarily relate with its expression and a phenotype of clinical resistance. However, a positive result for a genetic marker of antimicrobial resistance cannot be definitively linked to the microorganism detected, even if association is common. For example, in case of simultaneous detection of '*Klebsiella pneumoniae*' and '*KPC*' targets in setting where KPC-producing *K. pneumoniae* is endemic, there is a high probability of linkage between the targets revealed, but theoretically the KPC determinant could also be carried by another microorganism not detected by the molecular system because off-panel (e.g. *Providencia* spp.) or in-panel (e.g. *E. coli*) but under the detection threshold of $10^{3.5}$ copies/mL. Moreover, when multiple detection of microorganisms (e.g. '*Klebsiella pneumoniae*' and '*Proteus* spp.') and a genetic marker of antimicrobial resistance (e.g. 'KPC') occur, it is impossible to establish which is the correct combination pathogen/resistance gene based on molecular results. Even if these concepts are cumbersome to be outlined in a report, some efforts should be done to clarify the potential ambiguity of molecular results.

2.5. Clinical impact of rapid diagnostic tests for pneumonia

Several studies have highlighted the potential advantages of rapid molecular testing for pneumonia, in terms of improving patient management and antimicrobial stewardship strategies. A recent RCT investigated the role of rapid diagnostic test (RDT) for detection of MRSA in BAL versus standard method (quantitative culture) with the aim of reducing the use of unnecessary anti-MRSA coverage (vancomycin or linezolid) in ventilator-assisted patients with pneumonia [83]. High sensitivity (95.7%) and low negative likelihood ratio (0.04) of rapid PCR for MRSA, observed during the validation phase, supported the choice of early antibiotic discontinuation based on RDT results. Patients in intervention group received a shorter anti-MRSA empirical therapy (32 h [IQR 22–48] vs 72 h [IQR 50–113], $p < 0.001$) and a shorter anti-MRSA treatment [46 h (IQR 24–73) vs 122 h (66–219), $p < 0.001$] in 28 days after enrollment; they also had no increased duration of mechanical ventilation, ICU and hospital stay. Moreover, hospital mortality tended to be lower in the group managed with rapid PCR. A similar experience has been recently pursued in one of our Centers (Sant'Orsola-Malpighi Hospital in Bologna, Italy) through a prospective study of adult patients diagnosed with late HAP/VAP hospitalized from June to October 2018. Rapid PCR for MRSA on endotracheal aspirate or BAL obtained using investigational-use-only (IUO) FA-PPP was compared with culture method as the gold standard for etiological diagnosis of pneumonia. Results of (IUO) FA-PPP did not interfere with clinician decisions, so that patients were managed according to the standard of care (SOC). (IUO) FA-PPP sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for MRSA detection were 100%, 89%, 14%, and 100%, respectively. Positive and negative likelihood ratio were 9.5 and <0,1, respectively. These findings supported that FA-PPP could be useful in limiting exposure to anti-MRSA antibiotics in patients with HAP/VAP.

Regarding coverage for MDR Gram-negatives, Sant'Orsola-Malpighi Hospital has recently tested (IUO) FA-PPP vs SOC on respiratory samples (43 ETA, 15 BAL) of 58 consecutive patients hospitalized for HAP/VAP from July to December 2018. Of these, 27 had a prior rectal colonization with bla_{KPC}-harboring *K. pneumoniae*, while 31 were not colonized. Agreement between negative results were obtained in 17% of cases, while a false negative (IUO) FA-PPP result was observed in only one patient with *S. maltophilia* infection, which is an off-panel species. Concordant positive results for the same pathogen were 41%, arriving up to 53% after adding microorganisms with <10^4 CFU/ml; 43 new pathogens were found with FA-PPP in 27 cases, including fastidious microorganisms and polymicrobial infections. Regarding the detection of antimicrobial resistance markers, 35 positive tests matched SOC results, no false negatives were observed. Seven cases were considered 'false positive'; they consisted of 6 patients colonized with KPC producing *K. pneumoniae* who developed pneumonia with isolation from the respiratory sample of a susceptible Gram-negative pathogen and the remaining case was a non-colonized patient with FA-PPP positive for *K. pneumoniae* (10^7 CFU/ml), *S. aureus* (10^6 CFU/ml) and KPC marker, while culture was positive only for a susceptible *K. pneumoniae*.

RDT results should always be reviewed by skilled microbiologists and clinicians in order to optimize antibiotic therapy. A French observational study assessed (IUO) FA-PPP on different specimens (36 ETA, 27 spontaneous/induced expectorations) from 63 episodes of CAP/HAP, of which 70% (43/63) occurred in immunosuppressed patients and 59% (37/63) were managed in ICU. Then RDT results were independently reviewed by an infectious disease (ID) and an ICU physician who proposed the following therapeutic management accordingly. Based on ID specialist evaluations, antibiotic regimen could have been early escalated in 24% cases and de-escalated in 55% (vs 16% and 33% according to ICU physician), resulting in potential saving of up to 133 days of broad-spectrum antibiotic therapy. Authors concluded that a rapid identification of microorganisms matched with an expert evaluation could optimize the use of antibiotics [84]. Another recent multicenter analysis has tested (IUO) FA-PPP on 259 BAL of patients with suspected respiratory tract infections and compared it to standard methods (culture or PCR when required). (IUO) FA-PPP showed a positive and a negative concordance with SOC of 96% and 98% respectively. False negative results were reported in three cases where fastidious bacteria were cultured. SOC-/FA+ events were 84, of these 50% occurred in patients under effective antibiotic therapy within 72 h prior the sample collection and another 42% involved common oral flora. Multiple bacterial species were isolated by RDT in 32 cases; in 28/32 (88%) cases the microorganism considered predominant matched the predominant species found with SOC culture. Viral pathogens were detected by FA-PPP in 19% of BAL, but a SOC test had not been required for 77% of these RDT positive specimens. According to this study, change in antibiotic treatment could have been made on 68% (175/243) of patients tested with FA-PPP [85].

Other interesting insights concerning FA-PPP could be proposed. For instance, exploiting its potential ability to speed up the turnaround time, it could be possible to wait before administering empirical antibiotic therapy in stable patients without signs or symptoms of sepsis/septic shock, especially if a diagnosis other than bacterial pneumonia is suspected (i.e. viral or fungal infections, tuberculosis, cancer).

3. CONCLUSIONS

In conclusion, the development of rapid molecular tests has largely contributed to fasten the diagnosis of pneumonia, and implementation of these tests should be considered in the diagnostic workup of selected pneumonia patients. The introduction of these tests, which are complementary but not substitutive of conventional culture, mandates for a significant revision of the laboratory workflow. In this perspective, a multidisciplinary approach is fundamental because only the close cooperation between microbiologists, clinicians and an antimicrobial stewardship team can allow optimal positioning of the molecular tests, the correct interpretation of the results, and the most ambitious goal of making the best clinical choice as quickly as possible.

4. EXPERT OPINION

Conventional diagnosis of pneumonia based on quantitative and qualitative culture of pathogens is associated with a significant reduction in mortality, since it helps to establish a definitive and targeted antibiotic therapy. However, recovery of potential pathogens by culture requires a relatively long timeframe and sensitivity is influenced by several factors (i.e. antibiotic exposure prior to specimen collection, fastidious growth characteristics of some pathogens, or overgrowth of resident microbiota) that reduce the ability of conventional diagnostic microbiology to provide accurate and timely results. Consequently, antimicrobial treatment must be empirically started based on clinical presentation and other risk factors. Moreover, the diagnostic approach based on conventional culture does not detect viruses or atypical bacteria, which provide a relevant contribution to CAP.

Recently, molecular diagnostics have been introduced that provide a notable contribution to the rapid diagnosis of pneumonia, and diagnostic workflow incorporating these tests could make the difference in the quality of patient care [82]. Indeed, molecular diagnostics for pneumonia have suitable characteristics to assist clinicians in giving the 'right treatment' (by allowing detection with high sensitivity/specificity of the most prevalent pathogens and of several relevant resistance determinants), to the 'right patient' (e.g. high NPV for early interruption of antibiotics if negative result for bacterial infections) in a 'short time' (e.g. results rapidly available due to fast turnaround time).

However, the implementation of such molecular tests entails also some limitations and leaves some gaps, that make the cultural method still necessary.

Considering recent advances on lung microbiome alteration at the origin of pneumonia [28,86], the use of a pre-set molecular panel may miss the involvement of further bacterial or viral agents in pneumonia pathogenesis. Indeed, diagnostic based on cultures and whole genomic sequencing (WGS) seem to be more suitable. However, low yield of culture of species forming lung microbiota and the current limited use of WGS data - requiring high cost, additional time for analysis, and high-level bioinformatic expertise for its interpretation - may downgrade their role in clinical practice.

Another challenge of molecular diagnostics concerns interpretation and reporting of positive results, since the high sensitivity of such technologies could result in positive tests not confirmed by culture due to a low bacterial load (under the limit of detection of culture method), remnants of non-viable microorganism in the sample, or circulating nucleic acid of colonizing bacteria. All these aspects require a careful interpretation of positive results, that must be adequately conveyed to the clinician in a report as clear as possible and useful for the management of the patient. On the other hand, a negative result gives a crucial and conclusive information to clinicians, allowing to exclude the presence of major pathogens responsible for pneumonia and of clinically relevant resistance determinants, with a significant impact on the appropriate use of antibiotics (i.e. discontinuation or de-escalation) and eventually on the outcome of patients. In

this perspective, it would be desirable for comprehensive syndromic pneumonia panels to include additional important respiratory pathogens, such as *S. maltophilia, Aspergillus* spp., and *Pneumocystis jirovecii*., which are currently missing in most commercial syndromic panels, and emerging pathogens, such as SARS-CoV-2.

Another concern with molecular diagnostics is their correct positioning in the laboratory workflow according to the principles of antimicrobial stewardship. The extension of opening hours of laboratories (i.e. on a 24/7 schedule), together with the availability of rapid diagnostic tests in all the major healthcare facilities, appear to be mandatory interventions to timely provide accurate results and achieve the utmost benefits from these tests.

A final challenge is represented by the cost and relatively low processivity of the molecular tests, which require a careful selection of patients who could better benefit from molecular diagnostics to avoid waste of resources.

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